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Recombinant human respiratory syncytial virus (RSV) monoclonal antibody Fab is effective therapeutically when introduced directly into the lungs of RSV-infected mice

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ABSTRACT **Previously, recombinant human respiratory syncytial virus (RSV) monoclonal antibody Fabs were generated by antigen selection from random combinatorial libraries displayed at the tip of filamentous phage. Two such Fabs, which exhibited high binding affinity for RSV F glycoprotein (a major protective antigen), were evaluated for therapeutic efficacy in infected mice just before or at the time of peak virus replication in the lungs. Fab 19, which neutralized RSV infectivity with high efficiency in tissue culture, was effective therapeutically when delivered directly into the lungs by intranasal instillation under anesthesia. In contrast, RSV Fab 126, which failed to neutralize virus in cell culture, did not exhibit a therapeutic effect under these conditions. The amount of Fab 19 required to effect a 5000- to 12,000-fold reduction in titer of RSV in the lungs within 24 hr was rather small. In four separate experiments, a single instillation of 12.9–50 μg of RSV Fab 19 was sufficient to achieve such a reduction in pulmonary virus in a 25g mouse. The use of Fabs instead of the whole immunoglobulin molecules from which they are derived reduced the protein content of a therapeutic dose. This is important because the protein load that can be delivered effectively into the lungs is limited. The therapeutic effect of a single treatment with Fab 19 was not sustained, so that a rebound in pulmonary virus titer occurred on the 2nd day after treatment. This rebound in pulmonary RSV titer could be prevented by treating infected mice with a single dose of Fab 19 daily for 3 days. These observations suggest that human monoclonal Fabs grown in *Escherichia coli* may prove useful in the treatment of serious RSV disease as well as diseases caused by other viruses where replication *in vivo* is limited primarily to the lumenal lining of the respiratory tract.**

Antibodies specific for respiratory syncytial virus (RSV) are of importance because of the urgent need to prevent or treat disease caused by this virus. Indeed, RSV is the major cause of serious viral lower respiratory tract illness in infants and young children worldwide (1). This virus is estimated to be responsible for ≈1 million deaths annually among infants and young children, mostly in developing countries. In the United States and in this age group, RSV commonly causes serious lung disease, necessitating hospitalization and intensive medical care. This virus has also recently emerged as an important cause of fatal pulmonary disease among individuals of any age who are immunosuppressed incident to organ transplantation, cancer chemotherapy, or human immunodeficiency virus (HIV) infection.

Studies in experimental animals provided solid evidence that RSV-specific neutralizing antibodies could (*i*) prevent virus infection in the lungs when administered before exposure to virus and (*ii*) effect rapid resolution of infection when administered at the height of infection (2). Clinical trials have validated these aforementioned experimental observations. In a recently reported clinical trial, pooled human IgG, which contained a high titer of RSV neutralizing antibodies and was administered monthly by the intravenous route at a dose of 0.75 g/kg, was shown to be highly effective in preventing serious RSV lower respiratory tract illness in high-risk infants and children (3). In addition, in a separate clinical trial, a similar preparation of human IgG (2.0 g/kg) administered by the intravenous route was shown to have a therapeutic effect in infants and young children hospitalized for RSV lower respiratory tract disease (4).

Passive prophylaxis of RSV disease in high-risk infants, children, and immunosuppressed adults requires that whole IgG molecules be used whether they are administered as purified human IgG prepared from pooled human plasma or as human RSV monoclonal antibodies prepared in the laboratory from transformed cells in cultures that produce these antibodies with high efficiency. The use of whole IgG molecules in passive prophylaxis is dictated by the need to maintain a protective level of RSV antibodies in the blood for the interval that protection is required, generally 9 months to several years. Hence, whole IgG molecules, which have a half-life in blood of ≈21 days, are preferred over $F(ab')_2$ or Fab fragments of IgG, which have a very short half-life in blood.

However, the situation is different for therapy of RSV infection of the respiratory tract. A previous study in cotton rats in our laboratory indicated that RSV antibody $F(ab')_2$ fragments were essentially equivalent in therapeutic efficacy to the whole IgG molecules from which they were derived when treatment was administered directly into the lower respiratory tract (5). This suggested that RSV-specific $F(ab')_2$ fragments administered into the lungs by small-particle aerosol might prove useful in the therapy of serious RSV disease.

Topical delivery of RSV antibodies directly into the lungs has a major advantage over parenteral administration of antibodies for the therapy of RSV disease. Antibodies delivered by the former route are 80–160 times more effective in therapy, thereby decreasing the amount of antibodies required by a factor of 80–160 (6). A further reduction in amount of antibodies required for therapy can be achieved by using monoclonal rather than pooled polyclonal antibodies. In this instance, the total amount of IgG required for therapy is reduced by an additional factor of 25–50. This means that

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Abbreviations: RSV, respiratory syncytial virus; pfu, plaque-forming unit(s); FI, fusion inhibiting; HIV, human immunodeficiency virus.

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the total amount of antibodies required for treatment of RSV disease can be reduced by a factor of 2000–8000 when monoclonal antibodies are administered directly into the lungs for treatment of RSV infection vis-à-vis parenteral administration of polyclonal antibodies. Nonetheless, this amount of IgG comes close to the limit of protein that can be delivered efficiently and safely with existing aerosol technology. Clearly, any improvement in the therapeutic effectiveness of antibodies delivered topically will increase the clinical usefulness and utilization of this approach. The observation that human RSV monoclonal antibody Fab fragments are effective therapeutically when introduced into the lungs of mice shortly before or at the height of their RSV infection represents such an improvement. This will decrease the amount of protein needed for topical therapy and offer other advantages that should facilitate commercial development and clinical realization of topical immunotherapy of RSV disease.

MATERIALS AND METHODS

Human Monoclonal Antibody Fabs. Human antibody Fabs were produced from combinatorial libraries prepared in filamentous DNA phage using the pComb3 system described by Barbas *et al.* (7, 8). Fabs with high binding affinity for RSV F glycoprotein were concentrated and purified by affinity chromatography using an anti-Fab antibody column as described by Barbas *et al.* (8). Three separate preparations of RSV Fab 19 were used. The first preparation was used in the studies shown in Tables 1 and 2. The second preparation was used for studies shown in Tables 3 and 4. The third preparation was used for the study shown in Fig. 1. Because the neutralizing activity of the preparations varied when tested in cell culture, the amount of preparation number 2 or 3 administered to mice was normalized to be equivalent in potency *in vitro* to that of the first preparation. HIV-1 Fab 12 has been described (9, 10). This Fab has a high binding affinity for HIV-1 gp120 and neutralizes several strains with high efficiency. HIV Fab DL21 has a high binding affinity for HIV-1 gp120 but does not neutralize the infectivity of the virus (C.F.B. and D.R.B., unpublished data). In experiments in which a HIV Fab served as a control, the amount of HIV-1 Fab administered was the same as for RSV Fab 19.

Therapy Studies in Mice. Female 15- to 32-week-old BALB/c mice were used. These animals weighed ≈25 g each. Mice were inoculated intranasally with $10^{6.3}$ plaque-forming units (pfu) of RSV strain A2 contained in 100 μl of tissue culture medium. Inoculation was performed after mice had been anesthetized with methoxyflurane; under these conditions, materials inoculated intranasally are delivered directly into the lungs. At 3 or 4 days after virus inoculation, 100 μl of Fab suspension was instilled intranasally as a single dose under methoxyflurane anesthesia. This treatment was repeated for some mice on the next day or on each of the next 2 days. At various times after virus inoculation, mice from each treatment or control group were sacrificed and their lungs were harvested (11, 12). Four to six mice of each experimental group were tested at each time point. Lung homogenates were titrated for RSV by plaque assay on HEp-2 cells, which were maintained under 0.75% methylcellulose in Opti-MEM (GIBCO) containing 2% fetal bovine serum (free of RSV antibodies) at 37°C in a 5% CO_2/95% air atmosphere (12). Plaques were detected by the immunoperoxidase labeling procedure (11).

Assay of RSV Neutralizing and Fusion-Inhibiting (FI) Antibodies. Neutralizing activity of the Fabs was measured by complement-enhanced plaque reduction using HEp-2 cell cultures and the A2 strain of RSV (13). The titer of neutralizing activity was calculated as the highest dilution of Fab that reduced plaque number by 60%.

Assay of FI activity of Fabs was performed by using HEp-2 cell cultures. Cell monolayers in 24-well tissue culture plates were inoculated with a dilution of RSV A2 strain calculated to yield 50 plaques per well. After incubation at 37°C for 1 hr, the inoculum was removed and the cell cultures were washed once with phosphate-buffered saline and then overlaid with 0.75% methylcellulose in Opti-MEM, which contained 1:4 dilutions of human serum or Fab and 2% fetal bovine serum, which lacked neutralizing activity for RSV. Cultures were then incubated for 5 days at 37°C after which the overlay was removed and the monolayers were fixed in 80% methanol. Plaques were stained by the immunoperoxidase staining procedure (11). FI titers were expressed as the reciprocal of the highest dilution of serum or Fab that inhibited plaque size by 50% or more.

RESULTS

Isolation of RSV Fabs. Human RSV antibody Fabs were generated by antigen selection from random combinatorial libraries displayed on the surface of filamentous DNA phage (7–10, 14). RSV Fab 19 has been described and was selected from an IgG1κ phage display library derived from the bone marrow of a HIV-1-seropositive donor (8, 14). RSV Fab 126 was selected from an IgG1κ library generated from spleen RNA of an elderly donor who had died of heart failure. Both Fabs were selected by panning the respective libraries against RSV FG glycoprotein, a baculovirus-expressed, engineered chimera of the two major protective envelope glycoproteins of RSV. This chimera was kindly supplied by M. Wathen (Upjohn). Both Fabs exhibited high affinity ($\approx 10^8$ M^{-1}) for the F glycoprotein as judged by ELISA measurements. One RSV Fab (Fab 19, preparation number one) exhibited high neutralizing activity against the virus when tested by the plaque-reduction neutralization technique in HEp-2 cell cultures, whereas the other RSV Fab (Fab 126) did not appear to possess neutralizing activity in cell culture (Table 1). A third human monoclonal antibody Fab (Fab DL21), which was directed against the envelope glycoprotein gp120 of HIV-1, did not exhibit neutralizing activity against RSV in cell culture (Table 1). In an independent set of assays, the RSV Fab 19 was shown to also exhibit a very high FI activity. The FI titer of Fab 19 was approximately one-half that of its neutralizing antibody titer.

Therapeutic Efficacy of RSV Fab 19. The two RSV Fabs and the HIV-1 Fab DL21 were then tested in mice for therapeutic efficacy 3 days postinfection, which is 1 day prior to the height of RSV replication in the lungs. A varying amount of Fab was instilled into the respiratory tract of anesthetized mice 3 days after infection with RSV. Treatment with RSV Fab 19 (preparation number one), which exhibited a high level of neutralizing activity in cell culture, caused a significant reduction in the level of RSV in the lungs of BALB/c mice on day 4, which is when RSV ordinarily reaches peak titer. As little as 3.2 μg of Fab 19 (or 129 μg of Fab 19 per kg of body weight) was active therapeutically in mice. Mice given 12.9 μg (or 516 μg per kg of body weight) of Fab 19 exhibited a greater therapeutic effect in which the titer of

Table 1. *In vitro* neutralizing activity of three purified, concentrated human monoclonal antibody Fabs

Monoclonal antibody Fab	Fab, μg/ml	Reciprocal titer of neutralizing activity	Fab needed for 60% reduction of RSV plaque formation, μg
RSV 19*	258	1552	0.17
RSV 126	273	<10	≥27.30
HIV DL21	405	<10	≥40.50

*Preparation number one.

RSV in the lungs was reduced by a factor of 5000. In contrast, RSV Fab 126 or HIV-1 Fab DL21, which did not exhibit neutralizing activity against RSV in cell culture, also failed to reduce the titer of RSV in the lungs of infected mice. In addition, the RSV Fab 19 did not exhibit a therapeutic effect in mice infected with influenza A/Udorn/1972 virus, providing additional evidence for the specificity of the therapeutic effect of this Fab against RSV infection *in vivo* (Table 2).

Rebound in Virus Titer After Single Treatment with RSV Fab 19. Next, the duration of the therapeutic effect of Fab 19 against RSV infection was investigated by measuring the amount of RSV present in the lungs of mice at various times after intranasal instillation of the Fab 19 (preparation number 2) (Table 3). The ability of Fab 19 (25 μg per mouse) to cause a significant reduction in the amount of virus in the lungs 24 hr after treatment was confirmed (Table 3). However, 2 days later a rebound in virus titer was observed. Thus, on the 3rd day posttreatment (which was the 6th day postinfection) the titer of pulmonary virus in the Fab 19-treated mice did not differ significantly from that of the control groups—namely, mice given the HIV Fab DL21 or mice that did not receive any treatment.

Rebound in Virus Titer Is Abrogated by Three Sequential Daily Treatments with RSV Fab 19. The observations cited above suggested that successful therapy with Fabs might require more than a single treatment in order to suppress virus replication until recovery had occurred. The feasibility of this approach was investigated initially in a study with Fab 19 (preparation number 2) summarized in Table 4. One group of mice received RSV Fab 19 (25 μg per mouse) only on the 3rd day postinfection, another group was treated on the 3rd and 4th days postinfection, while the remaining group of mice received the Fab on the 3rd, 4th, and 5th days postinfection. As in the prior experiments, a single instillation of Fab 19 reduced pulmonary RSV in mice by a factor of 2500 but a moderate rebound to a higher level occurred 24 hr later. This rebound was less than that observed in the previous experiment (Table 3) so that the titer of virus, in the treated mice, was still reduced by a factor of 100 compared to the control mice. During the next 2 days, the titer of pulmonary virus in the treated mice did not approach the high level present at the height of RSV replication—i.e., $10^{6.5}$ pfu on day 4 postinfection. Instead, on the 6th and 7th days postinfection the titer of pulmonary virus in these treated mice remained at a level

Table 2. Intranasal administration of human RSV monoclonal antibody Fab 19 on day 3 postinfection causes a significant decrease in virus in the lungs of RSV-infected BALB/c mice

Fab administered on day 3	Fab dose, mg per kg of body weight	Virus titer in lungs on day 4	
		RSV subgroup A*	Influenza A†
RSV 19‡	0.516	2.4 ± 0.33	6.4 ± 0.25
	0.258	4.2 ± 0.47	ND
	0.129	4.8 ± 0.23	ND
	0.032	5.5 ± 0.09	ND
	0.008	6.0 ± 0.06	ND
RSV 126	0.548	5.6 ± 0.11	6.5 ± 0.29
	0.274	5.9 ± 0.12	ND
HIV DL21	0.60	5.9 ± 0.04	ND
None	—	6.1 ± 0.14	6.8 ± 0.08

ND, not determined.

*Animals were inoculated with $10^{6.3}$ pfu of RSV subgroup A strain A2 intranasally on day 0. Titers were calculated as $\log_{10}$ pfu per g of tissue (mean ± SE of six animals).

†Animals were inoculated with 10^6 tissue culture ID_{50} ($TCID_{50}$) influenza A/Udorn/1972 virus intranasally on day 0. Titers were calculated as $\log_{10}$ $TCID_{50}$ per g of tissue (mean ± SE of four animals).

‡Preparation number one.

Table 3. A single intranasal instillation of RSV Fab 19 reduces the titer of RSV in lungs of infected BALB/c mice below the level of detectability but is followed by rebound 2 days later

Antibody used to treat on day 3*	Virus recovery from lungs, $\log_{10}$ pfu per g of tissue			
	Day 4	Day 6	Day 8	Day 10
RSV Fab 19†	<1.7	4.8 ± 0.18	<1.7	<1.7
HIV Fab DL21	5.6 ± 0.12	4.9 ± 0.14	<1.7	<1.7
JEC serum	4.8 ± 0.10	4.3 ± 0.21	<1.7	<1.7
None	5.8 ± 0.07	5.1 ± 0.09	<1.7	<1.7

*On day 3 postinfection 25 μg of the indicated Fab or a 1:4 dilution of human RSV immune serum was administered intranasally in a 100-μl volume under light methoxyflurane anesthesia; human JEC serum had a titer of RSV neutralizing antibodies of 1:1728 as measured by plaque reduction against RSV strain A2.

†Preparation number two.

($10^{4.2}$–$10^{4.3}$ pfu) consistent with imminent resolution of infection—in other words, the titer was similar to that seen in untreated mice on day 7 (Table 4; Fig. 1*A*).

Treatment on 2 or 3 successive days caused an even greater reduction in pulmonary virus titer (Table 4). RSV could not be detected in the lungs of mice in the latter group 1 day after cessation of therapy, while a very modest rebound was observed 1 day later. This rebound did not appear to be significant because virus could be detected only in the lungs of two of four mice tested and the amount of virus recovered was still considerably less than in the control groups (Table 4).

The effect of successive daily instillation of RSV Fab 19 on the rebound of RSV titer in the lungs of infected mice was examined in greater detail in a study that extended from day 1 through day 10 postinfection. Unlike the previous study (Table 4) in which Fab 19 was first administered intranasally on day 3, initial instillation of the antibody fragment was delayed until the 4th day postinfection when virus replication was at its peak (Fig. 1). RSV Fab 19 (preparation number 3) (50 μg) was administered intranasally to anesthetized mice on day 4 postinfection (Fig. 1*B*) or on days 4, 5, and 6 postinfection (Fig. 1*C*). Pulmonary RSV titers were determined from day 1–10 postchallenge, an interval that encompassed the entire course of RSV infection in these animals. As in the previous experiments in which RSV Fab 19 was administered on day 3 postinfection, instillation of the antibody fragment on day 4 effected a marked reduction in titer of pulmonary RSV within 24 hr. This 20,000-fold reduction in pulmonary RSV titer was followed by a rebound the next day. However, the amount of virus in the lungs of mice treated with RSV Fab 19 only on day 4 (Fig. 1*B*) never subsequently attained the pulmonary RSV titer of the control untreated mice (Fig. 1*A*). Rebound was completely abrogated when RSV Fab 19 was also administered on 2 successive days after the initial treatment on day 4 postinfection (Fig. 1*C*).

Examination of Alternative Possible Mechanisms Responsible for the Reduction of Pulmonary RSV or Its Subsequent Rebound. We evaluated the possibility that the observed reduction in titer of RSV in the lungs was due to neutralization *in vitro* when the lung homogenates were prepared and assayed for infectivity in cell culture. Individual lung homogenates of four untreated, infected mice were prepared on day 4 postinfection (Fig. 1*A*) and each was mixed separately with an equal volume of lung homogenate derived from a different uninfected mouse that had received 50 μg of Fab 19 (preparation number 3) 24 hr previously. Plaque assay of the four mixed homogenates yielded essentially the same titer as the unmixed lung suspensions from the infected mice. The difference in geometric mean titer of the two groups was only $10^{0.1}$. This indicated that the therapeutic effect observed in Fab recipients was due to an action of the Fab *in vivo* rather

Table 4. Rebound of virus replication after administration of RSV Fab 19 on day 3 postinfection is reduced by additional daily instillations of the Fab

Fab used to treat mice	Day(s) of treatment	Virus recovery from lungs, $\log_{10}$ pfu per g of tissue			
		Day 4	Day 5	Day 6	Day 7
None	—	6.5 ± 0.10	6.0 ± 0.15	5.6 ± 0.10	4.6 ± 0.21
RSV Fab 19*	3	3.1 ± 0.25	4.0 ± 0.41	4.3 ± 0.34	4.2 ± 0.10
	3 and 4	—	2.4 ± 0.15	3.8 ± 0.12	3.3 ± 0.15
	3, 4, and 5	—	—	<1.7	2.0 ± 0.19
HIV Fab 12	3, 4, and 5	—	—	5.6 ± 0.21	4.2 ± 0.10

*Preparation number two.

than neutralization of virus *in vitro* during homogenization of lung tissue.

Finally, the possibility that emergence of neutralization escape mutants caused virus rebound was investigated. RSV isolates from the lungs of three separately infected mice that exhibited rebound of RSV titer 3 days after a single treatment with RSV Fab 19 (Table 3) were passaged once in HEp-2 cells, and the resulting virus suspensions were tested for the efficiency with which the viruses were neutralized by the RSV Fab. Each of the isolates was neutralized by Fab 19 with the same efficiency as the RSV suspension used to infect the mice (data not shown). Therefore, selection and emergence of neutralization escape mutants were not responsible for the rebound in the pulmonary RSV titer observed on day 6 in these mice.

DISCUSSION

Our observations suggest that RSV Fabs, such as Fab 19, may prove effective for treatment of serious RSV lower respiratory tract disease occurring in normal infants or the elderly as well as individuals of any age who are at high risk because of respiratory tract or cardiac anomalies or immunodeficiency incident to genetic disease, suppressive therapy for organ transplantation, or HIV infection. In addition, these observations suggest that direct respiratory tract administration of Fabs such as Fab 19 may also be effective for short-term prophylaxis of serious RSV disease in high-risk individuals who are exposed to infection during hospitalization or a visit to an outpatient clinic.

Although we had previously observed that human RSV polyclonal antibody $F(ab')_2$ fragments exhibited a high level of therapeutic efficacy against RSV infection in cotton rats, we were surprised that the RSV Fab 19 antibody fragment displayed a similar high therapeutic activity *in vivo*. Indeed, to our knowledge a therapeutic effect of antibody Fabs *in vivo* had not been reported previously. This therapeutic efficacy was observed despite the fact that Fabs lack two functions provided by whole antibodies that are potentially important for virus clearance. First, Fabs are monovalent and, therefore, cannot cross-link virus particles or molecules on the surface of an individual virion (15). Second, they lack the Fc portion of the IgG molecule and for this reason cannot mediate effector functions such as complement activation or antibody-dependent cell cytotoxicity. Nevertheless, the RSV Fab 19 was very active in reducing the amount of virus present in the lungs of RSV-infected mice. This may signal the beginning of an era of immunotherapy of mucosal virus infections, such as those caused by RSV as well as other respiratory tract viral pathogens such as the influenza viruses, the parainfluenza viruses, and the rhinoviruses whose growth *in vivo* is limited primarily to the lumenal lining of the respiratory tract.

The RSV Fab 19, which exhibited therapeutic efficacy against the virus *in vivo*, has an additional property that

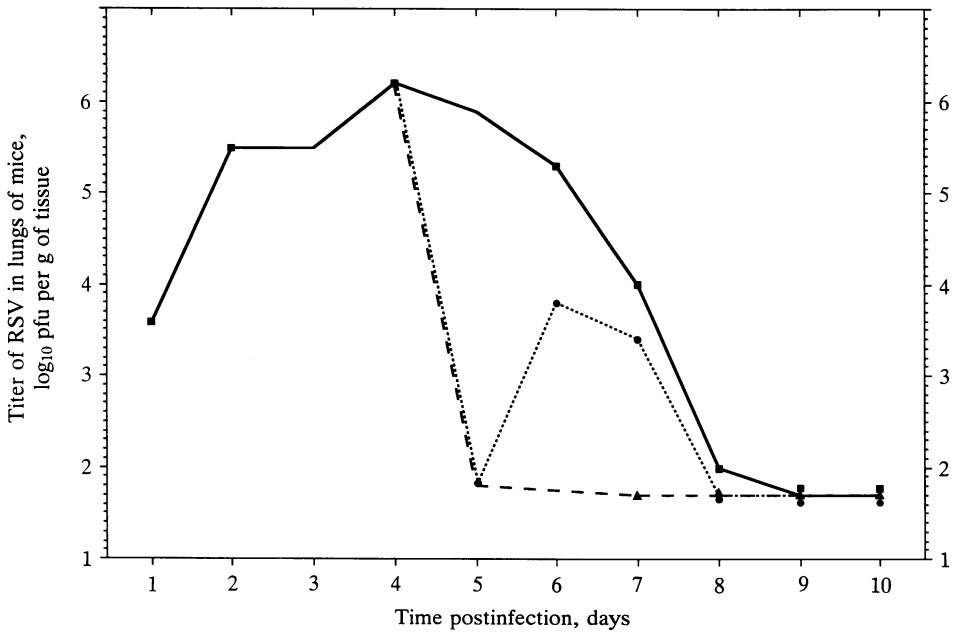

FIG. 1. Rebound of virus replication after administration of RSV Fab 19 on day 4 postinfection is reduced by additional daily instillation of the Fab. BALB/c mice were infected intranasally with $10^{6.3}$ pfu of wild-type RSV on day 0 and then were treated intranasally with 50 μg of RSV Fab 19 (preparation number three) on day 4 alone (●), on days 4–6 (▲), or were not treated (■). The titers of virus in the lungs of RSV-infected mice treated on days 4–6 with a control Fab (HIV-12) did not differ from that of untreated mice (data not shown). Four animals were sacrificed at each time point indicated in the figure, and lung tissue was obtained for determination of virus titer on the indicated day.

suggests that it may be useful in treatment of human RSV lower respiratory tract disease. Fab 19 is directed against a highly conserved site on the F glycoprotein of RSV strains of antigenic subgroup A and subgroup B (8, 14). Thus, in a previous study, Fab 19 neutralized each of 10 subgroup A and 9 subgroup B strains with high efficiency, although those viruses were isolated over a 30-year interval in widely diverse geographic locations (8, 14). Although we did not detect the emergence of neutralization escape mutants after treatment of RSV-infected mice with RSV Fab 19, it is likely that this would occur if a large number of patients were to be treated only with this antibody fragment. For this reason, it would be prudent to use a mixture of Fabs that include, in addition to Fab 19, one or more RSV Fabs that are active therapeutically and are directed against antigenic sites distinct from that seen by Fab 19. In this manner, the emergence of RSV antigenic escape mutants could be prevented.

Fabs offer several advantages over $F(ab')_2$s and whole IgG as a therapeutic modality. First, Fabs can be produced easily and inexpensively in bacteria, such as *Escherichia coli*, whereas whole IgG molecules require mammalian cells for their production in useful amounts. The latter entails transfection of IgG coding sequences into mammalian cells and the selection of stable, high-expression transformants. Rigorous selective procedures are needed for the selection and maintenance of such transformants. In addition, cell culture is required for production of whole IgG molecules by such transformants with the attendant problems of growth and maintenance of mammalian cells. In contrast, production of Fabs in *E. coli* obviates these difficulties and makes it possible to produce these antibody fragments in large fermenters with less expense than cell culture-derived products. Second, because Fabs have only one binding site for their cognate antigen, the formation of immune complexes is precluded, whereas such complexes can be generated when divalent $F(ab')_2$s and whole IgG molecules encounter their target antigen. This is of some importance because immune complex deposition in tissues can produce adverse inflammatory reactions. Third, Fabs lack an Fc region and hence cannot trigger adverse inflammatory reactions mediated by Fc, such as initiation of the complement cascade. Fourth, the tissue penetration of the small Fab molecule is likely to be much more efficient than that of the larger whole antibody.

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